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ANTI BACTERIAL EFFECT OF PIT AND FISSURE SEALANTS RELEASING FLUORIDE TREATED BY CHLORHEXIDINE (AN IN VIVO STUDY)

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ABSTRACT

Objectives: The aim of this study was to evaluate anti-bacterial effect of its and fissure sealants releasing fluoride treated by chlorhexidine. **Subjects and Methods:** The study included thirty patients. Group A: Molars that had pits and fissures sealed with fluoride-releasing materials. Molars in group B that had pit and fissure sealants applied release fluoride and CHX. Isolation of the Tooth, Acid Etching, Sealant Placement and Curing, and Sealant Evaluation Following Sealant Placement are all part of the treatment protocols for the two groups. Microbiological evaluation to determine the number of colonies of the S. mutant. **Results:** From baseline to one week, the mean percent reduction in streptococcus levels for group B was 34.06.6, while for group A it was 21.016.4. With a p value of 0.008, this was statistically significant and more prevalent in Group B. With a p value of 0.150, the mean percent drop in streptococcus levels from baseline to one month and three months was statistically insignificant. **Conclusion:** One percentage of chlorhexidine added to sealants have minimal to no impact on their mechanical qualities while having noticeable impact over the antibacterial activity of fluoride sealant.

KEY WORDS: fluoride, CHX S. mutans, pit and fissure sealants.

INTRODUCTION

Pit and fissure sealants have been suggested for caries prevention and are one of the most often utilized non-operative preventive techniques in dental health care. Fluoride treatment reduces tooth demineralization and promotes remineralization to prevent dental cavities [1,2]. Fluoride is applied in water fluoridation, toothpaste, mouthwash, dietary supplements, and professionally applied fluoride compounds like gels and varnishes. Fluoride can slow down the metabolism of bacteria found in dental plaque and speed up the remineralization of demineralized enamel [3].

After the germs are identified and the pit and fissure are sealed, only 50% of cariogenic bacteria, including Streptococcus mutans, remain [4]. Antibacterial agents can be added to the sealant material to increase the benefits of caries prevention [5]. CHX is one of the chemical antiplaque agents that is most frequently used to control oral bacteria. Bacteriostatic and bactericidal antimicrobial activities are shown by CHX digluconate. Numerous studies have shown that CHX is an antibacterial agent that effectively reduces the number of acidic bacteria in the mouth, which prevents the demineralization of the tooth surfaces. [6].

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The objective of this research was to determine the antibacterial effect of one percentage of CHX addition to pit and fissure sealants releasing fluoride. This study's null hypothesis was that varied releasing fluoride pits and fissure sealants with CHX had no effect on S. mutans biofilms.

PATIENT AND METHODS

Selection of patients

The sample population was selected from those patients referred by the clinic of pediatric and public health department, Faculty of Dental medicine Al-Azhar University with initial diagnosis free of carious and need preventive measurement to safe new erupted molars from decay due to dental caries, both male and female healthy patients, ranging from 6 to 14 years old.

Ethical Consideration

The Ethical Committee of the Faculty of Dentistry (Cairo, Boys), Al-Azhar University approved the conduct of this clinical study with regard to the Ethical approval code (546/2055).

Inclusion criteria

Patient age from 6 to 14 years' old. Young permanent molars with deep fissures. Vital teeth free from caries and any morphological disturbance.

Exclusion criteria

Uncooperative child, patient with carious or periodontally affected teeth.

Patients consent

After the initial assessment and acceptance, parents signed a written consent form after being fully informed of the process and duration of the therapy with an emphasis on the potential outcomes.

Grouping of patients

The study included thirty patients which was created as a randomized controlled clinical study with a maximum follow-up of three months. To ensure randomness all patient was given numbers from (1-30) then use GraphPad site (https://www.graphpad.com/) and programed it to make two random selected groups each group contain fifteen patient number as follows: Group A: Molars which receive pits and fissure sealants releasing fluoride Group B: Molars which receive pits and fissure sealants releasing fluoride with CHX

CHX mixing with pit and fissure sealant

Test specimens were prepared by adding 20% chlorhexidine digluconate (CHX) liquid in the following proportions: 0.05 ml of the CHX was pipetted and added to each 0.95 ml of resin sealant to adjust the concentration to 1% CHX. Content of the syringe (Fisseal, Promedica, Germany) sealant (2 ml) was placed into capsule wrapped by tin foil to avoid the sealant setting due to light. CHX and sealant was mixed together using amalgamator for 10 seconds to make sure to get homogenous mix. After mixing CHX and sealant the all the entire content in the capsule was reloaded to the sealant syringe. All this process was done in a dark room to prevent curing of the sealant [7].

Treatment protocol for both groups

Isolation of the Tooth: Isolation of the tooth was done by using light rubber dam. Clamp W8 was used in isolation of partially erupted first permanent molars. Clamp 202 and 208 were used in isolation of fully erupted first permanent molars.

Acid Etching: 37% phosphoric acid (gel) was applied by application tips

Visible Light Cured Sealant: Woodpecker RTA light cure (Woodpecker, China) was used in this study. Pits and fissure sealants were exposed for 30 second to the light cure.

Sealant evaluation after placement: Check the sealant for any pores, air bubbles, or material deficiencies after it has dried. By using the explorer to try to remove the sealant, the sealant retention was evaluated.

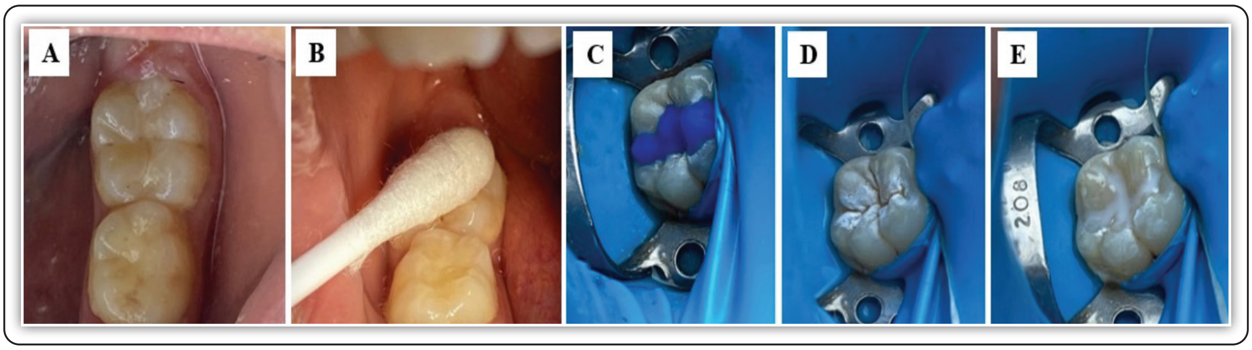

FIG (1) Photographs showing the pit and fissure sealant application procedure in case in group (A). (A) Pit and fissure of the tooth before application of pit and fissure sealant. (B) Taking the swab from tooth surface. (C) Acid etching of the tooth. (D) Chalky white appearance of the enamel. (E) Immediate placement of pit and fissure sealant.

Microbiological assessment:

First visit procedures: Swab was taken from the teeth surface of the patient using sterile swab (Pahrmatrix, India) before placement of the fissure sealant and swab was plated on Mitis Salivarius Agar (Sigma Aldrich, Germany) culture plates and incubated for 48 hours [(7)] to determine the numbers of streptococcus mutants colonies in the oral cavity.

Second visit procedures: After one-week swab was taken by sterile swab from the teeth surface to assess the numbers of streptococcus mutants in the oral biofilm.

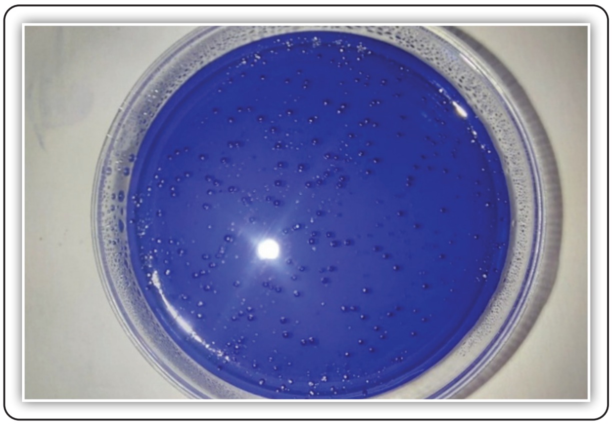

FIG (2) Photographs showing Streptococcus mutans colonies on Mitis Salivarius.

Third visit procedures: After one month the previous procedures were done to evaluate streptococcus mutants count in the oral biofilm.

Fourth visit procedures: After three months the previous procedures were done to assess the streptococcus mutants count in the oral biofilm.

RESULTS

Bacteriological outcome:

Streptococcus count /level:

The mean and standard deviation of Streptococcus count /level of both groups are presented in table (1). At the baseline (Before), the mean level of streptococcus was 1310±290 for group B and was 1156±227 for Group A (Fissure sealant). this was statistically insignificant with p value = 0.150. After one week, the mean level of streptococcus was 854±158 for group B and was 897±221 for Group A. This was statistically insignificant with p value = 0.557.

After one month, the mean level of streptococcus was 501±217 for group B and was 481±127 for Group A. This was statistically insignificant with p value = 0.783

After 3 months, the mean level of streptococcus was 220±119 for group B and was 177±64 for Group A. This was statistically insignificant with p value = 0.269.

Comparing mean level gradually in each individual group was statistically significant

($p<0.001$) and pairwise comparisons revealed that all time groups are statistically significant from each other's.

TABLE (1) Mean and SD of Streptococcus levels at different time points in the tested groups

	Group A		Group B		P value
Streptococcus	**Mean**	**SD**	**Mean**	**SD**	
Before	1156	277	1310	290	0.150
1Week	897	221	854	158	0.557
1Month	481	127	501	217	0.783
3Months	177	64	220	119	0.269
P value2	<0.001		<0.001		

P≤0.05 is statically significant; P1: for comparison between 2 groups by independent t test. P2: for comparison over time in each group separately by repeated measure ANOVA followed by Bonferroni post hoc test.

Streptococcus Levels percent reduction:

The mean and standard deviation of Streptococcus count /level percent reduction of both groups are presented in table (2). From baseline to 3 months, the mean percent reduction of streptococcus levels was comparable between groups with average 83%. This was statistically insignificant with p value = 0.974.

From baseline to 1 month, the mean percent reduction of streptococcus levels was 63.0±13.8 for group A(CHX) and was 54.7±14.9for Group B (Fissure sealant). This was statistically insignificant with p value = 0.150. From baseline to 1 week, the mean percent reduction of streptococcus levels was 34.0±6.6for group A(CHX) and was 21.0±16.4 for Group B (Fissure sealant). This was statistically significant with p value = 0.008; BEING higher in Group A

TABLE (2) Mean and SD of Streptococcus levels percent reduction between the tested groups

Streptococcus Percent reduction	Group A		Group B		P value
	Mean	**SD**	**Mean**	**SD**	
Before To 3 Months	83.8	4.9	83.6	8.1	0.974
Before To 1 Months	54.7	14.9	63.0	13.8	0.150
Before To 1 Week	21.0	16.4	34.0	6.6	0.008

P≤0.05 is statically significant; analysis done by independent t test

DISCUSSION

Pit and fissure sealants have been used to fill up pits and fissures, reducing the spread of plaque-causing bacteria [8]. It has been suggested that fluoride-releasing pit and fissure sealants may be able to stop dental caries, slow down enamel demineralization, and encourage remineralization[9].

The main microorganisms causing human dental caries have been identified as Streptococcus mutans[5]. S. mutans targets the tooth surface by producing calcium-depleting acids, but it also encourages other bacteria to cling to the tooth surface, accelerate demineralization, and increase plaque resistance[9]. Pit and fissure sealants have been used to fill up pits and fissures, preventing plaque buildup and cariogenic bacterial invasion. Pit and fissure sealants with fluoride-releasing capabilities have been proposed to have the ability to prevent caries, reduce enamel demineralization, and promote remineralization [9]. It has been demonstrated that chlorhexidine reduces plaque, gingival irritation, and bleeding [10]. It is considered to be a potent mechanical oral hygiene's adjuvant (brushing and flossing), particularly in situations where the patient was unable to do it in a right way. CHX is a harmless substance that is obtainable as mouthwash, gel, aerosol, spray, and discs. It has low and transient local and systemic negative effects [11].

In the current study, pairwise comparisons showed that all time groups differ statistically from one another when comparing the mean level of Streptococcus count / level across time in each individual group (p 0.001). The mean level of streptococcus for group A and group B at baseline (Before), after one week, after one month, and after three months was statistically insignificant. Both Group B and Group A experienced statistically insignificant mean percent reductions in streptococcus. In agree with Naorungroj et al. (12), three types of pit and fissure sealants were found to have antibacterial qualities. IN the agar diffusion assays, all compounds demonstrated either diffusible or contact antibacterial properties. According to the limitations of this in vitro experiment, it can be concluded that all substances have the ability to stop S. mutans and L. acidophilus from growing when they come into contact and in agree with with Shanmugaavel et al. (7) ,assess the impact of adding 1% chlorhexidine digluconate solution to glass ionomer and resin-based sealant on its mechanical characteristics and antibacterial activity. Both sealants' antibacterial properties were greatly improved by the addition of 1% chlorhexidine. In this current study, there is no significant different between two groups in bacterial reduction may be due to the one percentage of CHX addition was a low concentration while if the concentration of CHX was increased it might have an adverse effect in mechanical properties of pi and fissure sealants.

CONCLUSION

In summary, pits and fissure sealants releasing fluoride and addition of CHX to it had a proper effect on S. mutans. While there is no significant different between fluoride releasing pit and fissure sealants without and with addition of CHX in antibacterial effect.

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